REMARKS

Favorable reconsideration is respectfully requested in view of the following remarks. Applicants appreciate the courtesy shown by Examiner Mummert in discussing the case with Applicants' representatives on January 24, 2012. The discussions of the interview are reflected in the following remarks. Claims 1-7 are pending.

Information Disclosure Statement (IDS)

With regard to the "demandee", the "demandee" is interchangeable with the "defendant". That is, the "demandee" is responding to the demand for invalidity and defending the patent. Thus, the translation of the documents is correct.

Claim Rejections – 35 USC 103

Claims 1-5 are rejected under 35 USC 103(a) as being unpatentable over EP 0971039A2 (Rabbani et al.) in view of WO 96/001327 (David et al.). Applicants respectfully traverse the rejection.

Claim 1 recites a first primer that contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence (hereinafter, the first primer will be referred to as a "turn-back primer" or "TP"). Claim 1 further recites a second primer that contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other (hereinafter, the second primer will be referred to as a "folded primer" or "FP").

Advantageously, when the first and second primers according to claim 1 are used, a target gene can be amplified isothermally with high specificity. The advantageous

effects of claim 1 are demonstrated, for example, by Example 4 on pages 63-66 of the present specification.

In particular, Example 4 involves the use of two types of templates: (1) a region of a human STS DYS237 gene (hereinafter, "wild-type DNA"); and (2) the same region of the human STS DYS237 gene of (1) but having a single nucleotide mutation (hereinafter, "mutated-type DNA")(see pages 63-64 of the specification). Example 4 also involves the use of two types of primer sets: (1) a primer set for detecting the wild-type DNA (this primer set includes R1 and F1, which correspond to the first and second primers of claim 1, respectively); and (2) a primer set for detecting the mutated-type DNA (this primer set includes R1G and F1, which correspond to the first and second primers of claim 1, respectively). The only difference between the primer set for detecting the wild-type DNA and the primer set for detecting the mutated-type DNA is a single nucleotide in the sequence (B') of the first primer (the difference is at the fifth residue from the 5' end of R1 and R1G; see page 63 and Figure 8 of the specification). The sequence (B') as recited in claim 1, is a sequence on the primer that hybridizes to the complementary sequence (Bc) of the sequence (B) on the target nucleotide sequence.

The following isothermal amplification reactions were conducted. The amplified products were achieved in as little as one hour. The results are shown in Figure 9.

(1) An amplification reaction using the wild-type DNA as a template and the primer set for detecting the wild-type DNA (lane 2 in Figure 9).

(2) An amplification reaction using the mutated-type DNA as a template and the primer set for detecting the wild-type DNA (lane 3 in Figure 9).

(3) An amplification reaction using the wild-type DNA as a template and the primer set for detecting the mutated-type DNA (lane 4 in Figure 9).

(4) An amplification reaction using the mutated-type DNA as a template and the primer set for detecting the mutated-type DNA (lane 5 in Figure 9).

As shown in Fig. 9, amplification products were obtained in lanes 2 and 5, but not in lanes 3 and 4. Further, each of lanes 2 and 5 showed a band around 120 base pairs, which is the expected size of the correct amplified product. These results show that the primer set for detecting the wild-type DNA amplifies the wild-type DNA but does not

amplify the mutated-type DNA, and that the primer set for detecting the mutated-type DNA amplifies the mutated-type DNA but does not amplify the wild-type DNA. Thus, the experimental data of Example 4 clearly demonstrate that the primers in accordance with claim 1 allow highly specific amplification to be achieved such that even a single nucleotide mutation can be detected in a gene, and the highly specific amplification can be conducted efficiently.

Rabbani and David do not disclose or suggest the features of claim 1. The rejection contends that it would have obvious to use a reverse folded primer as taught by David with Rabbani's forward turn-back primer and achieve the feature of claim 1. Applicants respectfully disagree.

In particular, the rejection relies on Figure 4 and paragraph [0043] of Rabbani for motivation in combining the references. With regard to Figure 4 of Rabbani, this figure depicts the mechanisms involved when a reverse turn-back primer (FE') binds to the extended strand (CB'C'D'E'F'G') that is formed by the forward turn-back primer (CB'). Note that the formation of the extended strand (CB'C'D'E'F'G') by the forward turn-back primer (CB') is depicted in Figure 1 of Rabbani. Figure 4 ① of Rabbani shows the reverse turn-back primer (FE') binding to the extended strand (CB'C'D'E'F'G') formed by the forward turn-back primer (CB') and Figure 4 ② shows the extension by the reverse turn-back primer (FE') along the extended strand (CB'C'D'E'F'G') formed by the forward turn-back primer (CB'). Thus, it is clear that the extended strand (CB'C'D'E'F'G') formed by the forward turn-back primer (CB') cannot be considered a primer.

The rejection appears to concede that the extended strand (CB'C'D'E'F'G') formed by the forward turn-back primer (CB') is not a primer, but contends since the structure of the extended strand (CB'C'D'E'F'G') looks similar to that of a strand extended by the folded primer, one would have been motivated to use a reverse folded primer as taught by David with the forward turn-back primer (CB') as taught by Rabbani.

However, as illustrated on page 8 of the Technical Explanation that was provided during the interview (for the Examiner's convenience, a copy is attached), the CB'C' portion of the extended strand (CB'C'D'E'F'G') shown in Figure 4 ① does not have the

same structure as a folded primer. That is, the complementary sequence of the CB'C' portion includes the sequence B to which another forward turn-back primer (CB') hybridizes, as shown in Figure 2 ④ of Rabbani. The folded primer does not have an intervening sequence corresponding to sequence B or B' to which another turn-back primer hybridizes. Thus, the CB'C' portion of the extended strand (CB'C'D'E'F'G') is not only different structurally, but also completely different in terms of functionality from the folded primer.

Moreover, even if the extended strand (CB'C'D'E'F'G') shown in Figure 4 ① could be considered as a forward primer, the strand (CB'C'D'E'F'G') and the reverse turn-back primer (FE') shown in Figure 4 ① would not be expected to successfully amplify a target sequence due to the formation of a dimer by the binding of the reverse turn-back primer (FE') to the strand (CB'C'D'E'F'G').

With regard to paragraph [0043], the rejection relies on paragraph [0043] for motivation of using a reverse primer that is structurally different from the forward primer, but paragraph [0043] specifically refers to paragraph [0042] in describing the "second primer" (paragraph [0043] specifically indicates the following: "In other aspects of the just-described process, the initial primer or nucleic acid construct and the second primer or nucleic acid construct can be the same, or they can be different"; the "just-described process" refers to the process described in paragraph [0042]). Paragraph [0042] is reproduced below (the relevant portions are underlined):

> **[0042]** This invention provides for a process to amplify linearly a specific nucleic acid sequence of interest that one seeks to amplify. Such a process includes the step of providing the following components and reagents: the specific nucleic acid sequence of interest, an initial primer or a nucleic acid construct comprising two segments, and appropriate substrates, buffer and a template-dependent polymerizing enzyme. The two segments of the initial primer or nucleic acid construct include (A) a first segment having two defined characteristics. First, it is (i) substantially complementary to a first portion of the specific nucleic acid sequence and second, it is (ii) capable of template-dependent first extension. The second segment (B) has four defined characteristics. First, the second segment (B) is (i) substantially non-identical to the first segment. Next, it is (ii) substantially identical to a second portion of the specific nucleic acid sequence. Third, the second segment (B) is (iii) capable of binding to a complementary sequence of the second segment. Fourth, this second segment is (iv) capable of providing for

> subsequent binding of a first segment of a second primer or nucleic acid construct to the first portion of the specific nucleic acid sequence under isostatic or limited cycling conditions. In so doing, a second primer extension is produced and that displaces a first primer extension. Another important step of this linear amplification process is that of incubating the specific nucleic acid sequence and the novel primer or nucleic acid construct in the presence of the appropriate substrates, buffer and template-dependent polymerizing enzyme under isostatic or limited cycling conditions; thereby linearly amplifying the specific nucleic acid sequence of interest that one seeks to amplify.

That is, paragraph [0042] makes clear that each of the "initial primer" and the "second primer" has a first segment that binds to the same template sequence. In other words, both the "initial primer" and the "second primer" are forward primers, and in paragraph [0043], Rabbani indicates that the initial forward primer and the subsequent forward primer can be the same or different. Rabbani does not disclose or suggest in paragraph [0043] that the forward primer and a reverse primer can be different.

In view of the above discussion, it is clear that one skilled in the art would not have been motivated to use a reverse folded primer as taught by David with Rabbani's forward turn-back primer in light of Figure 4 and paragraph [0043] of Rabbani.

Furthermore, even if there were a basis to consider using the folded primer (hereinafter, "FP") of David with the turn-back primer (hereinafter, "TP") of Rabbani, there would not have been a reasonable expectation of success in combining the references and achieving isothermal amplification with superior specificity and efficiency, as demonstrated, for example, by the experimental work of the specification.

Specifically, in general, successful amplification using a primer set that includes the TP depends on both the forward and reverse primers being functional. That is, even if one of the primers is functional, the success of the amplification is controlled by the non-functional primer. This is demonstrated, for example, by the experimental work provided in the enclosed Rule 132 Declarations (one by Mr. Yasumasa Mitani and another by Mr. Takefumi Ishidao). The Declaration by Mr. Ishidao includes information from the invalidation trial previously of record in "Prosecution documents of Mukou (Invalidation) 2008-800293" from the IDS filed on March 3, 2010.

The experimental results in the Declarations show that when both the forward and reverse primers are TPs, successful isothermal amplification can be achieved, but when the forward primer is a standard PCR primer (i.e., a primer that includes only a sequence that hybridizes to the template), and the reverse primer is a TP, successful isothermal amplification cannot be achieved.

Specifically, in Confirmatory Experiment 1 in the Declaration by Mr. Ishidao, primer pair 3 is a primer set where the forward and reverse primers are TPs (in the Declaration, the forward and reverse primers being TPs is indicated as "TP + TP"; see page 3 of the Declaration). The isothermal amplification results using primer set 3 is provided in lane 6 of Figure 3 in the Declaration by Mr. Ishidao (see page 8 of the Declaration for Figure 3; see also the top of page 4 of the Declaration for the legend of Figure 3). As shown in lane 6 of Figure 3, successful isothermal amplification was obtained using primer set 3 (TP + TP).

In the Declaration by Mr. Mitani, primer pair 2 is a primer set where the forward primer is a PCR primer and the reverse primer is a TP (in the Declaration, the forward primer being a PCR primer and the reverse primer being a TP is indicated as "PCR primer + TP"; see page 2 of the Declaration). The isothermal amplification results using primer set 2 is provided in lanes 4 and 5 in Figure 3 of the Declaration by Mr. Mitani (see page 4 of the Declaration for Figure 3; see also page 3 of the Declaration for the legend of Figure 3). As shown in lanes 4 and 5 in Figure 3, amplification products were not obtained using primer set 2 (PCR primer + TP).

The above results clearly indicate that successful isothermal amplification is controlled by the non-functional primer.

Further, David teaches the use of FPs for the forward and reverse primers, but the reference does not provide any experimental data demonstrating that successful amplification can be achieved using FPs for both the forward and reverse primers. In fact, as demonstrated in the experimental work in the enclosed Declaration by Mr. Ishidao, successful isothermal amplification cannot be achieved where FPs are used for both the forward and reverse primers.

Specifically, in Confirmatory Experiment 2 in the Declaration by Mr. Ishidao, forward and reverse FPs were prepared as shown in Figures 7 and 8 (see page 10 of the Declaration for Figure 7 and 8). The isothermal amplification results using the forward and reverse FPs shown in Figures 7 and 8 are provided in lanes 2-4 in Figure 9 (see page 10 of the Declaration for Figure 9; see also page 6 for the legend of Figure 9). Lane 2 shows the amplification results where a DNA polymerase that is incapable of strand displacement (Taq DNA polymerase) is used while lanes 3 and 4 show the amplification results where a DNA polymerase that is capable of strand displacement (Aac DNA polymerase) is used. As shown in lanes 2-4 of Figure 9, amplification products were not obtained using the forward and reverse FPs shown in Figures 7 and 8.

Thus, given the fact that successful isothermal amplification using a primer set that includes TP is controlled by the presence of a non-functional primer, and that successful isothermal amplification cannot be achieved using FPs for both the forward and reverse primers as taught by David, there would not have been a reasonable expectation of success in using David's reverse FP with Rabbani's forward TP and obtaining successful isothermal amplification, much less obtaining successful isothermal amplification with superior specificity and efficiency, as demonstrated, for example, by the experimental work of the specification. Accordingly, claim 1 and its dependent claims are patentable over Rabbani and David.

Claims 5-7 are rejected under 35 USC 103(a) as being unpatentable over Rabbani in view of David as applied to claims 1-5 above, further in view of Pastinen et al. (Genome Research, 1997, vol. 7, pp. 606-614). Applicants respectfully traverse the rejection.

Claim 1 has been distinguished above from Rabbani and David. Pastinen does not remedy the deficiencies of Rabbani. Claims 5-7 depend from claim 1, and are patentable over Rabbani, David and Pastinen for at least the same reasons discussed above. Applicants do not concede the correctness of the rejection.

In view of the foregoing, favorable reconsideration in the form of a notice of allowance is requested. Any questions or concerns regarding this communication can be directed to the attorney-of-record, Douglas P. Mueller, Reg. No. 30,300, at (612) 455.3804.

Respectfully submitted,

HAMRE, SCHUMANN, MUELLER & LARSON, P.C.
P.O. Box 2902
Minneapolis, MN 55402-0902
(612) 455-3800

Dated: Feb. 8, 2012

By: _____
Douglas P. Mueller
Reg. No. 30,300

DPM/ym

Technical Explanation

US Application 10/583,706

Dr.Yoshihide
Hayashizaki

24.01.2012

Claim 1 of the present application

(TP-FP primer set)

A primer set comprising at least two primers that allows a target nucleic acid sequence to be amplified,

wherein a **first primer** included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence, and

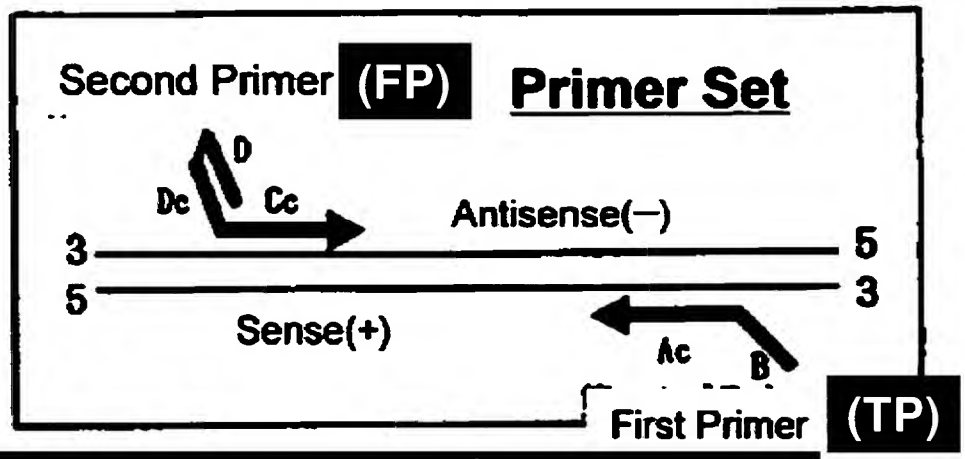
a **second primer** included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

*The first primer is **TP**, the second primer is **FP**.

TP; Turn-back Primer

FP; Folded Primer

Technical explanation of the TP and FP

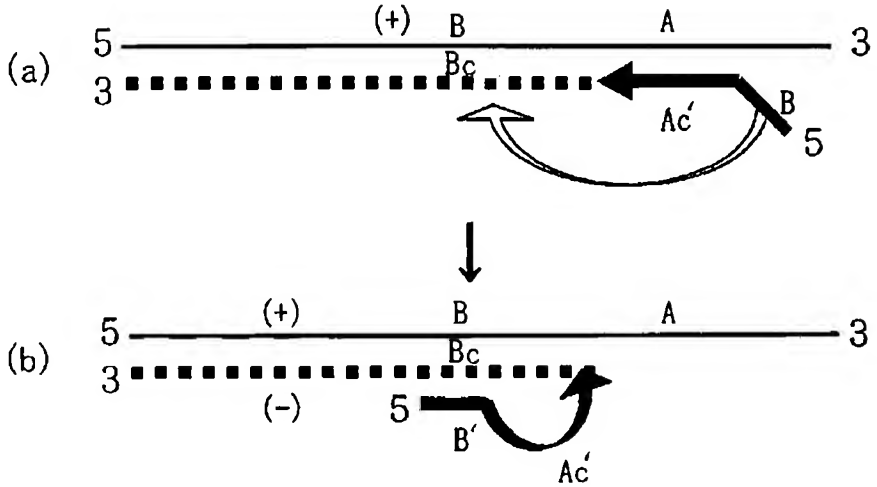


FP functions as follows:

(1) FP has the folded sequence (D-Dc') in the 5' side sequence.
(2) The folded sequence (D-Dc') has two nucleic acid sequences that hybridize to each other.
(3) The folded sequence (D-Dc') **DOES NOT** hybridize to the elongation strand from FP (**NO turn back**).
(4) **NO** primer hybridizes to the folded sequence (D-Dc') of the FP or the elongation strand from FP.

TP functions as follows:

(1) TP has the turn back portion (B) in the 5' side sequence.
(2) The turn back portion (B) can hybridize to the portion (Bc) of the elongation strand from TP.



Mechanism of the amplification reaction of the TP-FP(1)

(FIG.3 of the present invention)

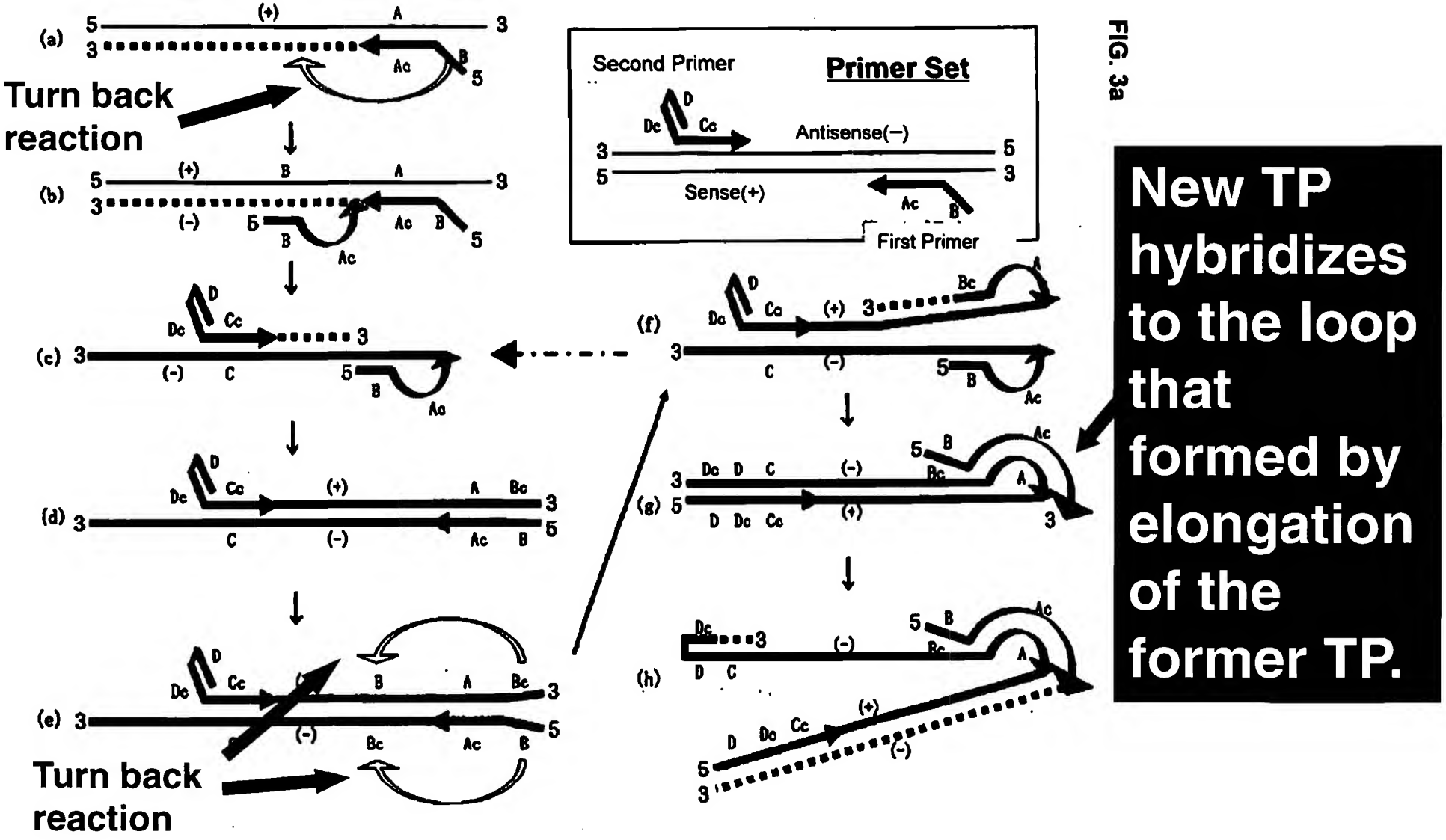


FIG. 3a

Mechanism of the amplification reaction of the TP-FP(2)

(FIG.3 of the present invention)

The characteristic feature of the FP itself or the folded portion formed by elongation of the former FP is that these portions do NOT hybridize to any primers. This feature enables us to prevent background amplification.

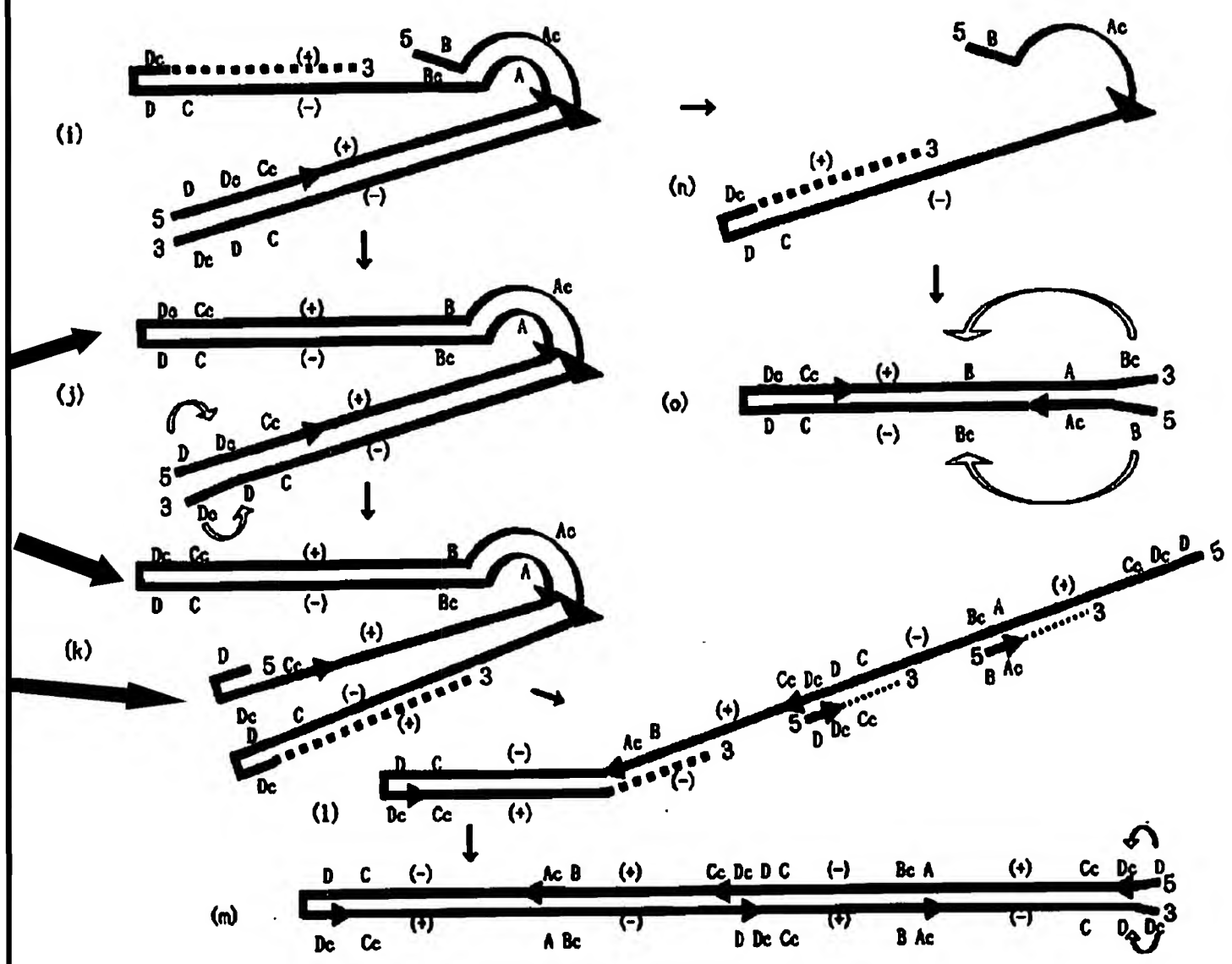


FIG. 3b

Summary of the Office Action

The Office Action explained the following:

(1) TP are shown in Figure 4, step 1 and 2 (① and ② shown in below) in Rabbani (EP0971039A2).

(2) Rabbani teaches that second primer can be different from initial primer (paragraph 43, p. 8).

(3) FP are shown in Fig. 5b in David (WO96/001327).

(4) A portion CB'C' in Figure 4 ① in Rabbani matches quite well with FP (Response to Arguments).

(5) Therefore, Claims 1 to 7 of the present invention are obvious (103(a)).

FP-like portion pointed out by the examiner.

TP pointed out by the examiner.

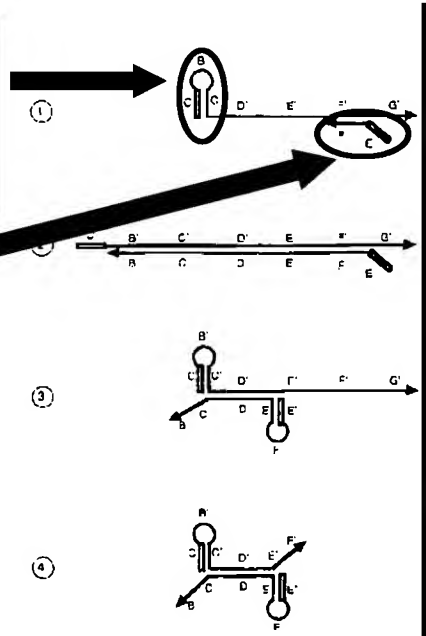


FIGURE 4

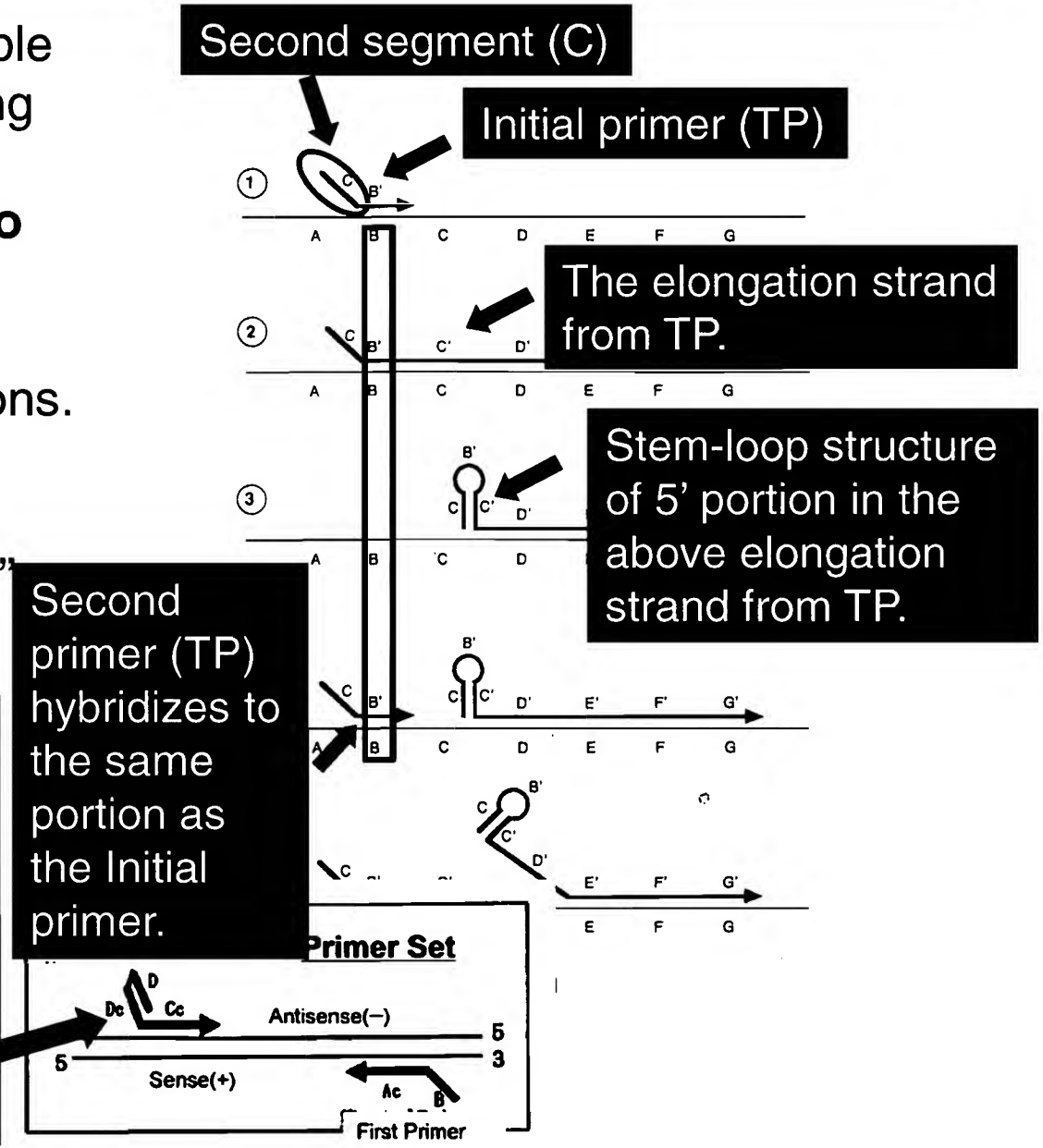
With regard to (2), second primer is NOT reverse primer (FP).

With regard to (4), the portion CB'C' in Figure 4 ① in Rabbani does NOT match folded sequence (D-Dc') of FP.

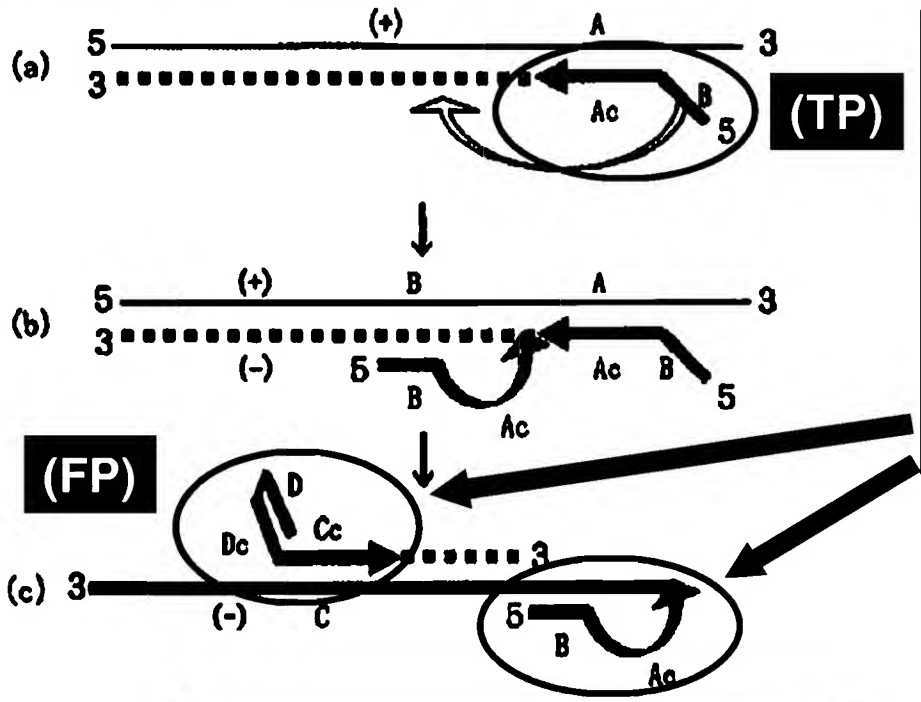
"Second primer" in Rabbani [0043] is NOT reverse (antisense) primer.

"this second segment is (iv) capable of providing for subsequent binding of a first segment of a **second primer** or nucleic acid construct **to the first portion of the specific nucleic acid sequence** under isostatic or limited cycling conditions. In so doing, a second primer extension is produced and that displaces a first primer extension." (Rabbani p8 l37~39 [0042])

"second primer" in Rabbani [0043] is NOT reverse (antisense) primer (different from the "second primer" in the present invention).



FP quite different from the stem-loop structure of elongation of TP (1).

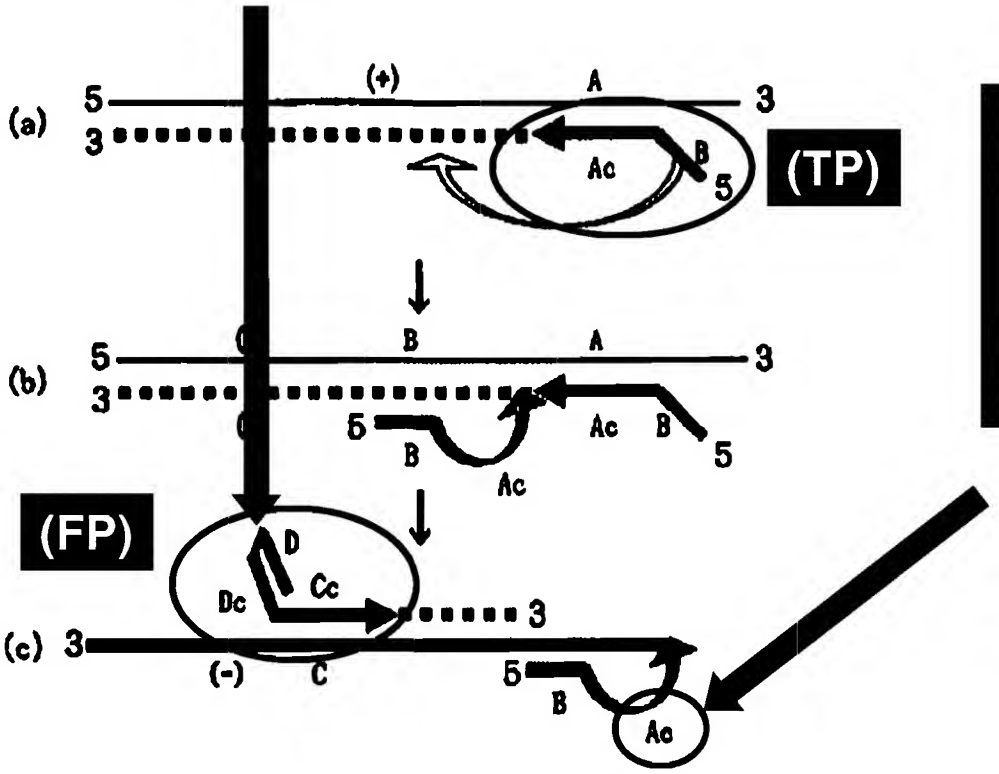


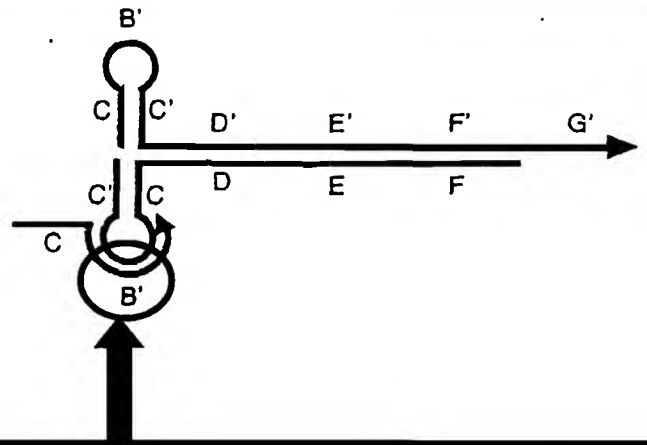
Stem-loop structure in elongation of TP

FP different in shape and function to the stem-loop structure in elongation of TP.

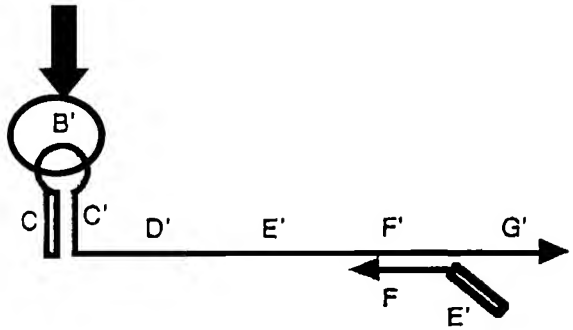
FP quite different from the stem-loop structure of elongation of TP (2).

FP does NOT have a sequence to which new primer hybridizes.

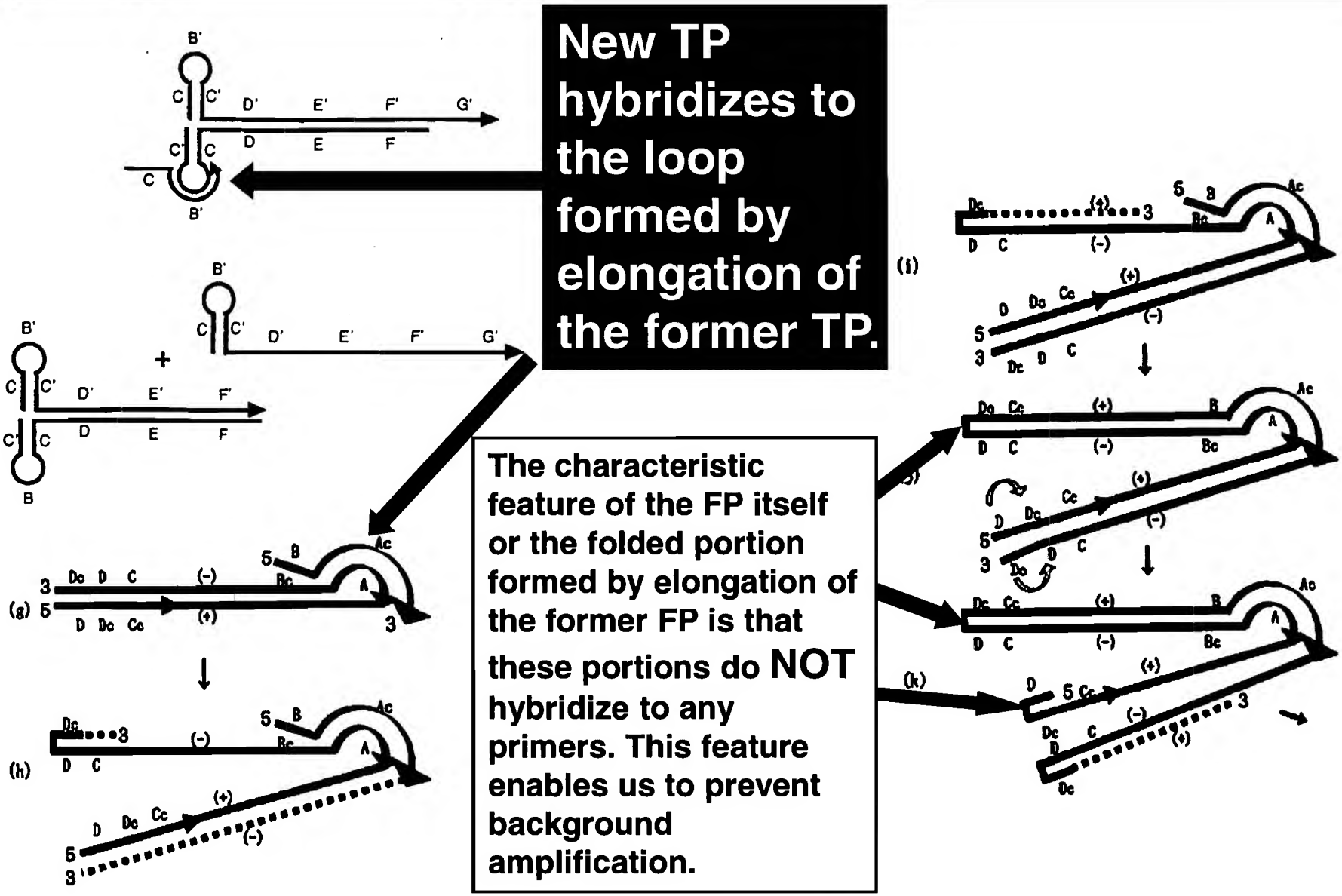




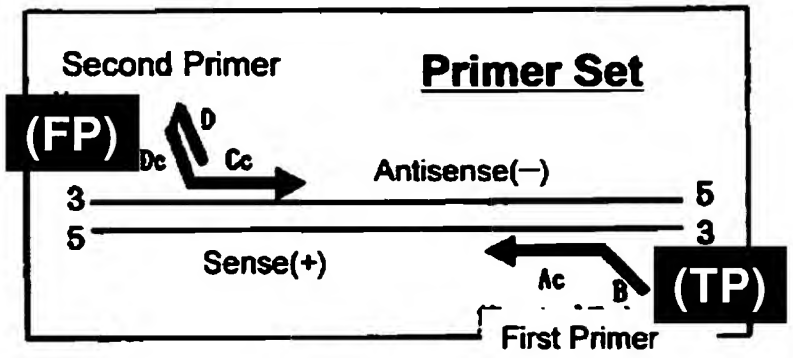
Stem-loop structure in elongation of TP has a sequence to which new TP hybridizes (FIGURE 2 ④ and FIGURE 4 ① in Rabbani, Fig. 3a (c) in the present application).

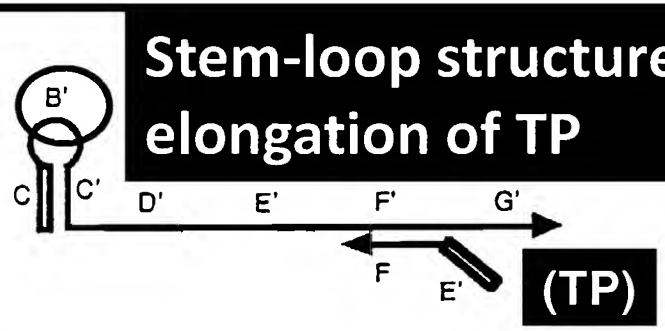


FP quite different from the stem-loop structure of elongation of TP (3).



Conclusions





(1) Stem-loop structure in elongation of TP (FIGURE 4 ① in Rabbani) is **different** in shape and function to FP.

(2) "Second primer" in Rabbani [0043] is a subsequent which hybridizes to the same portion to the initial primer, and **NOT** reverse (antisense) primer.

(3) So, a person of ordinary skill in the art is **NOT** motivated to reach the present invention from Rabbani and David.

Unexpectable advantages of the present invention by using TP and FP .

(1) Isothermal amplification

- The amplification occurs without thermal denaturation.

(2) Specific amplification

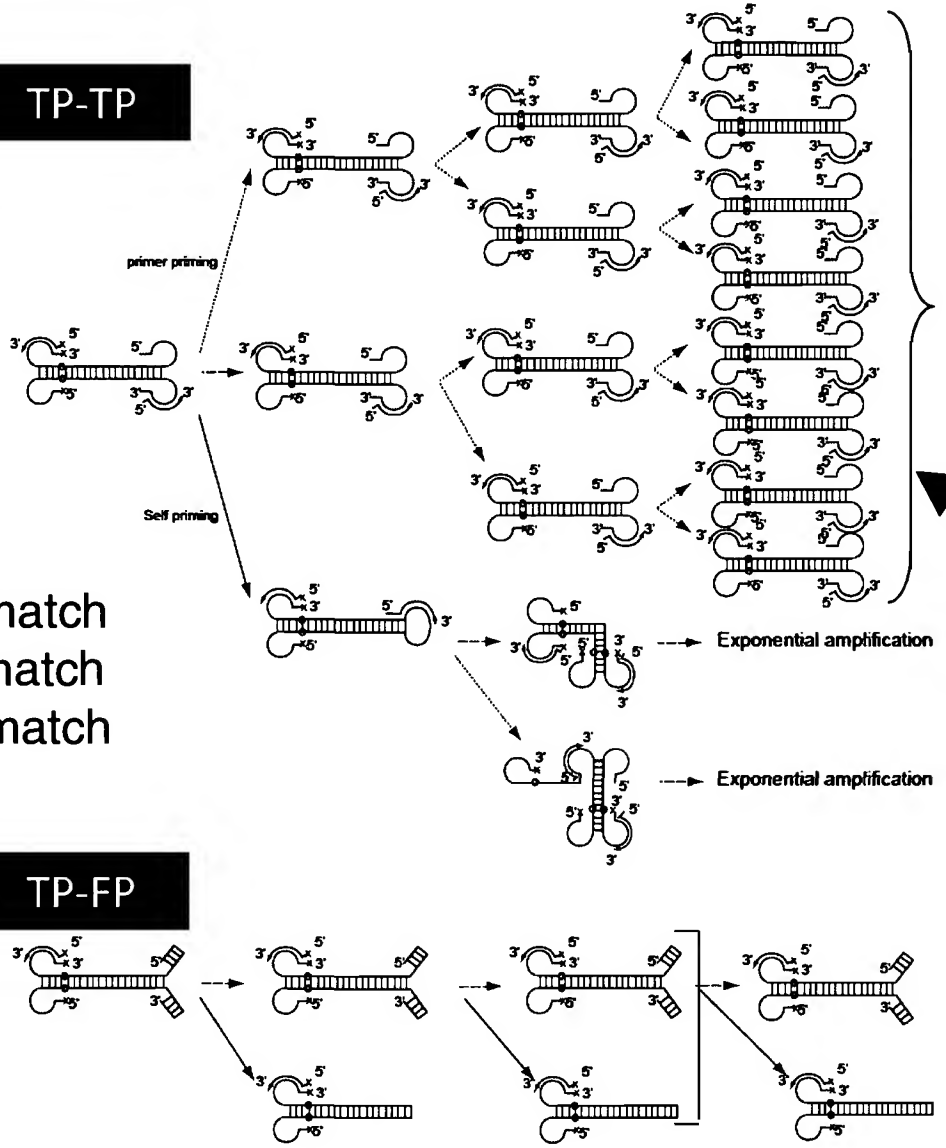
- The present invention can detect SNPs without non-specific amplification.

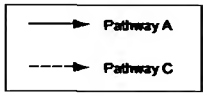
(3) Short time amplification

(4) Easy primer design

Mechanism of specific amplification (advantage (2))

The non-specific amplification **DOES NOT** occur in the present invention (TP-FP).





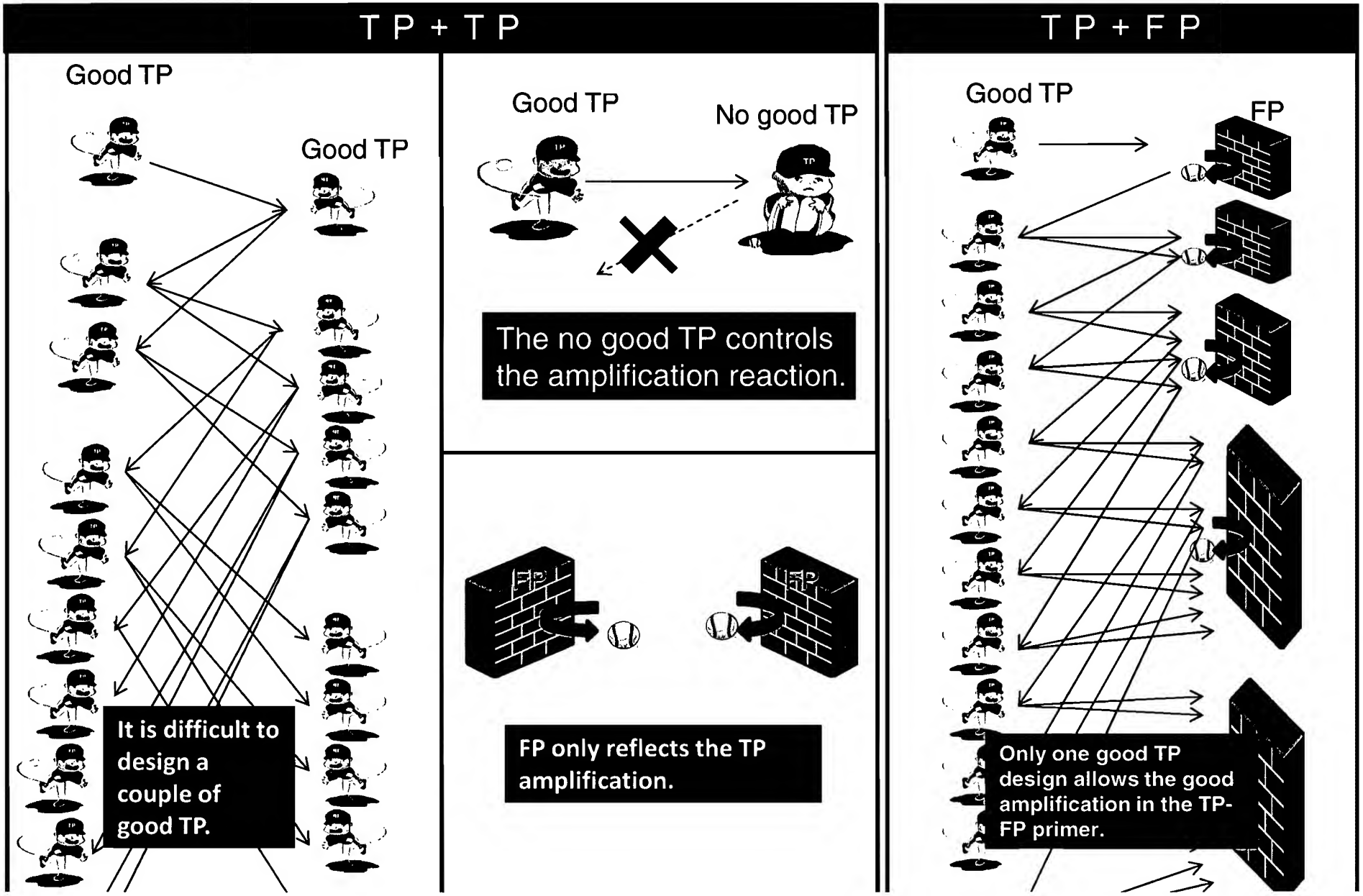
(1) TP-TP primer set **CAN NOT** detect SNPs.
(2) Because, TP-TP primer set has the pathway of the non-specific extensively exponential amplification (background amplification).

Exponential amplification

●•••X mismatch
●••••● full match
X•••X full match

(1) TP-FP primer set **CAN** detect SNPs.
(2) Because, the non-specific exponential amplification of the pathway of TP-FP primer set is very gentle.

Short time amplification (advantage (3))



Easy primer design(advantage (4))

(1) TP

(i) TP can amplify exponentially.

(ii) TP has a strong engine of amplification.

(iii) TP has two areas based on template sequence.

(2) FP

(i) FP can not amplify exponentially, but amplifies linearly.

(ii) FP is like a mirror which reflects TP amplification.

(iii) FP needs only one area based on template sequence.

(3) TP-TP primer set

(i) TP-TP primer set needs four areas based on the template sequence.

(ii) TP-TP Primer set needs the design of a couple of good TPs because the reaction is totally controlled by the presence of no good TP.

(iii) TP-TP Primer set is difficult to design.

(4) TP-FP primer set

(i) TP-FP primer set needs only three areas based on the template sequence. Thus, the distance (the number of bases) between TP and FP in TP-FP primer set can be made shorter than that between TP and TP in TP-TP primer set.

(ii) TP-FP Primer set needs the design of only one good TP because FP whose folded sequence can be designed in advance independently from template sequence **DOES NOT** control the reaction.

(iii) TP-FP Primer set is easy to design.